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Testes asymmetry of *Bufo gargarizans* in relation to body condition and age

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Abstract. Testes asymmetry theory predicts that males in good condition or mature age may be able to develop larger degrees of directional asymmetry than males in poor condition or younger age. In this study, we tested the testes asymmetry theory in the *Bufo gargarizans*. We found that *B. gargarizans* has a significant directional asymmetry in testes mass, with left testis being significantly larger than right testis. Male body condition was correlated with relative testes mass and absolute testes asymmetry, but not correlated with relative testes asymmetry. Therefore, we suggested that males in good body condition have the higher ability of sperm competition than males in poor body condition. Additionally, male age was correlated with absolute testes asymmetry, but not correlated with relative testes mass and testes asymmetry, thus not supporting the hypothesis that males with a higher degree of directional asymmetry survive better.

Keywords. Testes asymmetry, body condition, age, *Bufo gargarizans*.

Testes asymmetry theory predicts that directional asymmetry should be the norm because in many cases only one of the two testes is functional, with the other test having a compensatory role (Schärer and Vizoso, 2007). In this case, males in good condition or relatively older age may be able to develop larger degrees of directional asymmetry than males in poor condition or small age (Møller, 1994; Birkhead et al., 1997; Graves, 2004).

Directional testes asymmetry has been previously observed in birds (Birkhead et al., 1997) and mammals (Yu, 1998). Interestingly, in recent years similar results have been observed in some amphibians (Hettyey et al., 2005; Zhou et al., 2011; Liu et al., 2011; Liu et al., 2012). The Asiatic Toad (*Bufo gargarizans*) is widely distributed in East Asia, including China and portions of the Russian Far East, but is relatively rare on the Korean Peninsula. These toads breed during late-winter in large ponds or paddy fields (Yu and Guo, 2013), while overwinter in the sand or ponds. This species avoids dense forests, and is associated with open habitats such as grasslands, open forests, and cultivated areas. They prefer humid areas, and seldom live at altitudes of more than 800 meters (Yu and Guo, 2013).

However, we know little about testes asymmetry of *B. gargarizans* in relation to body condition and age. In this study, we examined whether the directional testes asymmetry occurs in *B. gargarizans*. Moreover, we tested whether positive correlations existed between degree of directional testes asymmetry, body condition and age.

Data were collected in vegetable fields of Nanchong city (30°35'N, 104°45'E; 300 m above sea level) in southern Sichuan, China. A total of 56 male toads were collected by hand at night in early-May, early-June, and early-September 2006, because the testis masses does not significantly change during the post-breeding season (i.e., after May) in the site (reviewed by Liu et al., 2011). Then, we took them to the laboratory and put those toads individually in a circular bucket (15L) with fresh water of 15 cm deep at room temperature for one night.

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Snout–vent length (SVL) of each captured toad was measured using a plastic ruler to the nearest 1 mm, and its body mass was measured using an electronic balance to the nearest 0.01 g. Then, 56 male toads were killed by double-pithing. We dissected the toads and took out their two testes, weighing them to the nearest 0.1 mg. Following Møller and Swaddle (1997) and Liu et al. (2011), we used two measures of testes to confirm degree of directional testes asymmetry: 1) absolute testes asymmetry, mass of the left testis minus the mass of the right (Liu et al., 2011); 2) relative testes asymmetry, the difference in testis mass / 0.5 (left-testis + right-testis mass) (Liu et al., 2011; Liu et al., 2012).

We used paraffin section and Ehrlich's haematoxylin stain to produce histological sections of the phalanges. Age was determined by counting the number of lines of arrested growth (LAGs) in the sections. This technique has been shown to be a reliable tool for determining age for a variety of amphibian species (Smirina, 1994), including *Rana chensinensis* (Lu et al., 2006), *Bufo andrewsi* (Liao and Lu, 2012).

Schulte-Hostedde et al. (2001) suggested that the measurement of live animals' condition is typically done by regressing body mass on body size and the residuals of this regression are used as an index of body condition. Body condition was calculated using linear regression by entering body mass as a dependent variable and SVL as independent variable, and the residual mass was used as an index. Similarly, relative testes mass was defined by entering testes mass as a dependent variable and SVL as independent variable using linear regression, and residual mass was used as an index. We used paired t-test to evaluate difference in masses of the left and right testis. We performed Pearson correlations to analyse the relationship between male age and body size or body mass. The relationships between male condition (dependent variable) and absolute testes asymmetry or relative testes asymmetry or relative testes mass (independent variable) were analyzed by linear regression analysis. Also, we used linear regression to test the relationships between male age (dependent variable) and absolute testes asymmetry or relative testes asymmetry or relative testes mass (independent variables). Statistical tests were performed by the software SPSS 14.0 for Windows (Statistical Product and Service Solutions Company, Chicago). All values are reported as mean ± SE and all statistical tests were two-tailed.

Average SVL and body mass of males were 8.09 ± 0.17 cm and 79.78 ± 4.64 g, respectively. Males had mean age of 2.32 ± 0.11 years (range: 1 to 4 years). Age was correlated significantly with male SVL (Pearson's correlation coefficient: $r = 0.83$, $n = 56$, $P < 0.001$) and body mass ($r = 0.94$, $n = 56$, $P < 0.001$).

Testes mass of *B. gargarizans* did not vary significantly during the post-breeding season (one-way ANOVA: $F_{2, 53} = 2.48$, $P = 0.09$). The testes mass of males showed directional asymmetry (Paired t-test: $t = 3.17$, df = 55, $P = 0.002$), with the left testes being significantly larger than the right ones in 71.43% of all the samples. The degree of absolute testes asymmetry was significantly correlated left testis mass with ($r = 0.51$, $n = 56$, $P < 0.001$).

We regressed body mass on male SVL, showing a highly significant regression ($F_{1, 54} = 398.13$, $R^2 = 0.881$, $P < 0.001$, body mass = 25.78×SVL (cm) − 128.796). Male condition was significantly correlated with the degree of absolute testes asymmetry (body condition = 0.21×absolute testes asymmetry − 1.522, $F_{1, 54} = 4.94$, $P = 0.03$; $R^2 = 0.084$, Fig. 1A), but not related to relative testes asymmetry ($F_{1, 54} = 0.99$, $P = 0.32$; $R^2 = 0.018$, Fig. 1C). Relative testes mass significantly increased in males with better body condition (body condition = 0.044×relative testes mass (mg) - 0.051, $F_{1, 54} = 5.77$, $P = 0.02$; $R^2 = 0.097$, Fig. 1E).

Male age was significantly correlated with the degree of absolute testes asymmetry (age = 0.026×absolute testes asymmetry + 2.14, $F_{1, 54} = 19.28$, $P < 0.001$; $R^2 = 0.263$, Fig. 1B), but not related with relative testes asymmetry ($F_{1, 54} = 1.44$, $P = 0.23$; $R^2 = 0.026$, Fig. 1D). There was also no significant correlation between age and relative testes mass ($F_{1, 54} = 0.02$, $P = 0.88$; $R^2 = 0.00$, Fig. 1F).

Our study revealed that the left testis mass was significantly larger than the right in the *B. gargarizans*. This result was similar to the pattern of testes asymmetry in other anuran species (*Rana nigromaculata*, Zhou et al., 2011; *Hylarana guentheri*, Liu et al., 2011; *Rana omeimontis*, Liu et al., 2012), while male frogs from all populations in *Rana temporaria* have larger right testes (Hettyey et al., 2005). Additionally, we found a significant positive relationship between the mass of the left testis and the degree of absolute testes asymmetry. This result was consistent with the hypothesis that left testes only are functional, and the right testis would increase in size if the left testis became nonfunctional (Møller, 1994; Birkhead et al., 1997). Testes asymmetry might occur because of multiple reasons. For instance, maintaining two large, functional testes may be costly (Møller, 1994), and one testis may have higher efficiency in sperm production due to constraints during embryonic development (Kempenaers et al., 2002).

Previous studies have suggested that testis asymmetry may not be a good measure of male body condition (Birkhead et al., 1997). Our results showed that male

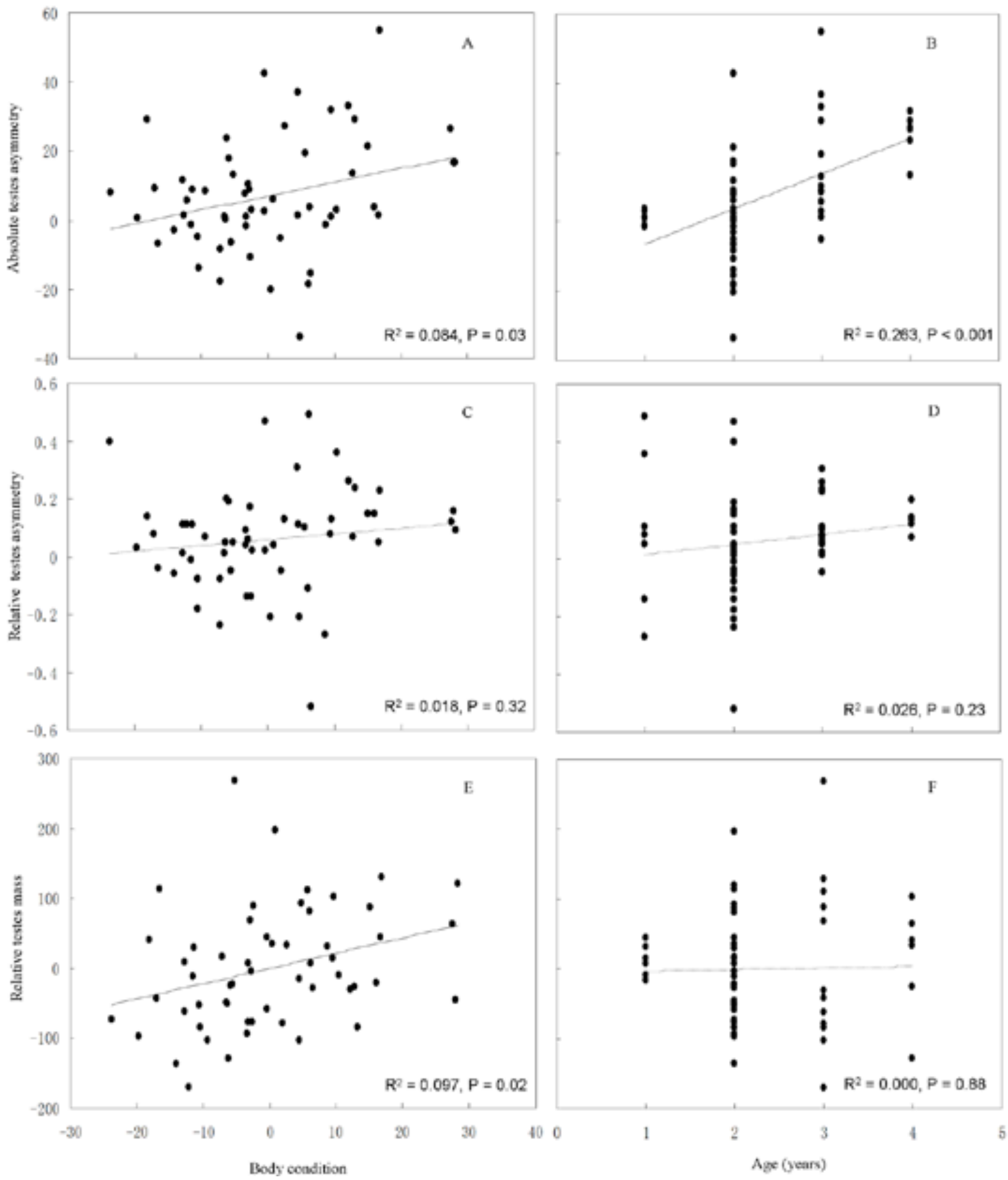

Fig. 1. Relationships between (A) body condition and absolute testes asymmetry, (B) age and absolute testes asymmetry, (C) body condition and relative testes asymmetry, (D) age and relative testes asymmetry, (E) body condition and relative testes mass, (F) age and relative testes mass. Displayed values are untransformed data for easier interpretation.

body condition was correlated with the degree of absolute testes asymmetry in *B. gargarizans*, but not correlated with the degree of relative testis asymmetry, suggesting that large males in good body condition had not a larger degree of testis asymmetry than small males in poor condition. This result was not consistent with Hettyey et al. (2005) and Møller (1994). Interestingly, male body condition was positively correlated with relative testis mass (Simmons and Kotiaho, 2002), suggesting that males in good body condition have the higher ability of sperm competition than males in poor body condition. Game theory models predict that ejaculate investment should increase with the risk and intensity of sperm competition (Parker, 1998). The testes should thus be relatively large when the likelihood of sperm competition is high (Møller and Briskie, 1995). Consequently, males in good conditions are expected to have larger testes than males in poor condition with increased risk of sperm competition (Olsson et al., 1997).

Previous studies have shown that testes asymmetry is age-related (e.g., Birkhead et al., 1997; Graves, 2004; Liu et al., 2012), suggesting that males with a higher degree of directional asymmetry survive better. However, we did not found age was positively correlated with the degree of relative testes asymmetry and relative testes mass. Liu et al. (2011) and Zhou et al. (2011) observed similar results in *Hylarana guentheri* and *Rana nigromaculata*, suggesting that older males did exhibit a higher degree of testis asymmetry than younger males. A possible explanation was males have already attained full reproductive maturity during the first breeding year (reviewed by Liu et al., 2011).

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REFERENCES

Birkhead, T.R., Buchanan, K.L., Devoogd, T.J., Pellatt, E.J., Szèkely, T., Catchpole, C.K. (1997): Song, sperm quality and testes asymmetry in the sedge warbler. Anim. Behav. **53**: 965-971.

Graves, G.R. (2004): Testicular volume and asymmetry are age dependent in black-throated blue warblers (*Dendroica caerulescens*). Auk **121**: 473-485

Hettyey, A., Laurila, A., Herczeg, G., Jönsson, K.I., Kovács, T., Merilä, J. (2005): Does testis weight decline towards the Subarctic? A case study on the common frog, *Rana temporaria*. Naturwissenschaften **92**: 188-192.

Kempenaers, B., Peer, K., Vermeirssen, E.L.M., Robertson, R.J. (2002): Testes size and asymmetry in the tree swallow *Tachycineta bicolor*: A test of the compensation hypothesis. Avian Sci. **3**: 115-122.

Liao, W.B., Lu, X. (2012): Adult body size = f (initial size + growth rate× age): explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. Evol. Ecol. **26**: 352-364.

Liu, W.C., Huang, Y., Liao, Y.M., Li, C. (2012): Testes asymmetry of Chinese endemic frog (*Rana omeimontis*) in relation to body condition and age. North-West. J. Zool. **8**: 390-393.

Liu, Y.H., Liao, W.B., Zhou, C.Q., Mi, Z.P., Mao, M. (2011): Asymmetry of testes in Guenther's Frog, *Hylarana guentheri* (Anura: Ranidae). Asian Herpetol. Res. **2**: 234-239.

Lu, X., Li, B., Liang, J.J. (2006): Comparative demography of a temperate anuran, *Rana chensinensis*, along a relatively fine elevational gradient. Can. J. Zool. **84**: 1789-1795.

Møller, A.P. (1994): Directional selection on directional asymmetry: Testes size and secondary sexual characters in birds. Proc. Roy. Soc. Lond B **258**: 147-151.

Møller, A.P., Briskie, J.V. (1995): Extra-pair paternity, sperm competition and the evolution of testis size in birds. Behav. Ecol. Sociobiol. **36**: 357-365.

Møller, A.P., Swaddle, J.P. (1997): Asymmetry, developmental stability, and evolution. Oxford: Oxford University Press.

Olsson, M., Madsen, T., Shine, R. (1997): Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. Proc. Royn. Soc. Lond. B **264**: 455-459.

Parker, G.A. (1998): Sperm competition and the evolution of ejaculates: Towards a theory base. In: Birkhead T.R., Møller A.P. (Eds.), Sperm Competition and Sexual Selection. San Diego, CA: Acad. Press, 3-54.

Schärer, L., Vizoso, D.B. (2007): Phenotypic plasticity in sperm production rate: there's more to it than testis size. Evol. Ecol. **21**: 295-306.

Schulte-Hostedde, A.I., Millar, J.S., Hickling, G.J. (2001): Evaluating body condition in small mammals. Can. J. Zool. **79**: 1021-1029.

Simmons, L.W., Kotiaho, J.S. (2002): Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. Evolution **56**: 1622-1631.

Smirina, E.M. (1994): Age determination and longevity in amphibians. Gerontology **40**: 133-146.

Yu, T.L., Guo, Y.S. (2013): Differential response to abiotic conditions, predation risk, and competition determine breeding site selection by two anuran species. Anim. Cells Syst. **17**: 127-132.

Yu, Z.H. (1998): Asymmetrical testicular weights in mammals, birds, reptiles and amphibia. Int. J. Androl. **21**: 53-55.

Zhou, C.Q., Mao, M., Liao, W.B., Mi, Z.P., Liu, Y.H. (2011): Testis asymmetry in the dark-spotted frog *Rana nigromaculata*. Herpetol. J. **21**: 181-185.